

December 7, 1957

Dear Dr. McQuillen:

Thank you for your note of Nov. 30.

As you may know, I have only just returned from a several months' trip to Australia. During my absence, I had left instructions to have sent you a copy of a manuscript detailing our work on *E. coli* L forms— as my assistant is not certain this was done, I am sending a duplicate under separate cover. This was to have been a partial answer both to the sentiments of your letter of Nov. 24, 1956 (as you can see I dug this out immediately) and your postcard last spring.

I am sorry to have appeared so irresponsible. If I can reconstruct the grounds for an apology, partly your question about terminology seemed rhetorical, partly I had not made up my own mind about the matter, and mainly I was too deeply involved in experiments that might have helped illuminate an answer.

Your criticism about the characterization of protoplasts seems to me entirely valid, and it's becoming more and more certain that different treatments are giving units with varying degrees of impairment of wall structure and function.

My only doubt is whether the most constructive approach to this question is on the semantic side. My first inclination on reading about protoplasts was that this was a horrid name to begin with, that its etymology did not at all convey the structural relationship "protoplast" + "wall" = "cell". But usage takes precedence over esthetics, and particularly in a rapidly growing field, we may not always know enough about our material to be sure that any classification will remain a valid one. For example, in Weibull's original report, he had to rely almost entirely on criterion a), but I doubt he should have been criticized on these grounds. And what will you do if (forfend it!) you find that some element of the cell wall (defined, say, by your present preparative technique) is retained on the surface of your protoplasts?

Nevertheless, I would be quite happy to see some more expressive term adopted for the genus that includes the various modes of wall-defect. I have had too notorious a neologophilia to be the appropriate author of such a generic term, though any of the following that appealed to you would be acceptable to me: (habro-, lepto-, malaco-, clado- etc., even sphero-) + plast or cyte. [Indeed the mammalian spherocyte does show a comparable pathology!]. I don't think that "protoplast" (in inverted commas) is the answer to this, though it does have one virtue that should be stressed. The scientific community is (as your proposed note suggests) incredibly careless about semantic usage, and particularly in the understanding of generic terms. I would rather fear that if say "spheroplast" were adopted, it would come to connote the alternative class of "false protoplast", rather than the inclusive class that needs to be cited.

Perhaps I have too confused an idea of wall structure, but still another way of looking at this problem is as follows: to my mind, "wall" connotes rigidity, shape, etc. If we translated this to chemical terms, we might choose to define the wall as that element which conferred these attributes—on your own argument, this would be the mucopolysaccharide. By this definition, the lipotein is not part of the wall, but, if you like, an elaboration of the plasma ~~inner~~ membrane, or another layer altogether. [It does not affect the argument, though it complicates the purification, if these layers are not simply concentric]. I am not seriously propounding this approach, but it is no less arbitrary than any other.

In summary, I think we are in probable agreement in our basic picture of organization of the cell, and in the desirability of a more generally expressive term, ~~xxxxx~~ corresponding to "protoplast". As there is likely to remain some doubt as to whether some existing, and many future, examples of wall defect will conform to each one of your criteria for a wall, plus any others that may occur to other workers, we need a descriptive term for the striking loss of wall function implicit in your (a). The inclusiveness, under such a term, of the lysotype-protoplasts of Bacilli, should be stressed.

To speak to a smaller detail, if I read you correctly, you will not accept any present claims of liberation of protoplasts from gram-negative bacteria. (It would be some comfort to me if you stressed that generalization in your account.) The reason for the experimental difficulty would be that the walls of these bacteria contain a lipoprotein component which is lacking in the gram-positives—perhaps this could be digested away from penicillin- or dap-deprived ~~pl~~ "protoplasts".

The ms. I am sending you (again?) has an account of "protoplast" liberation and L-form growth of DAP-less mutants. We are actively looking for auxotrophs relevant to other wall components: a large number of these (all those so far characterized) have been new DAP-less mutants; some other strains with less complete blocks, or with temperature-dependent blocks are responding to some complex materials, and are being run down now. Our trials for specific supplements should include: D- amino acids; glucosamine, galactosamine; DAP; and muramic acid, in addition of course to the usual run of familiar growth factors. We are also including galactose, rhamnose, fucose, etc. Would you have any other suggestions, particularly if coupled with a sample of the material in question or directions to the same?

Yours sincerely,

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Professor of Medical Genetics